THE EFFECT OF BLUEBERRIES ON LIPID PARAMETERS OF OVARIECTOMIZED RATS

By

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NOMENCLATURE

AHA	American Heart Association
BB	Blueberry
BMI	Body mass index
CHD	Coronary heart disease
CRP	C-reactive protein
CVD	Cardiovascular disease
HDL	High density lipoprotein
HRT	Hormone replacement therapy
LDL	Low density lipoprotein
Lp(a)	Lipoprotein a
OVX	Ovariectomy
Sham	Sham operated
TC	Total cholesterol
TG	Triglyceride
VLDL	Very low density lipoprotein
WHI	Women's Health Initiative
WHIMS	Women's Health Initiative Memory Study

CHAPTER I

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide and in the United States. It is estimated that 17 million people worldwide and over 927,000 Americans die each year from CVD (Centers for Disease Control and Prevention, 2005; World Health Organization, 2006). While CVD is the leading cause of death in both men and women, there are a greater number of females who die of CVD. In 2004, there were 459,096 females compared to 353,100 males in the United States who died from CVD (American Heart Association, 2007).

An elevated blood cholesterol level is a well known risk factor for developing CVD (Centers for Disease Control and Prevention, 2005; National Heart Lung and Blood Institute, 2006). The risk for CVD drastically increases for women when they reach menopause partly due to an elevation of total cholesterol levels after menopause. After menopause, total cholesterol levels increase putting women at a high risk for developing heart disease. On average, menopause occurs when women are between 45 and 55 years of age (World Health Organization, 1996). Women at this same age have also been shown to have a higher percentage of total cholesterol than men of the same age (Ford et al., 2003).

There are medications which have been approved to help control blood cholesterol levels but these medications can have unwanted side effects (National Heart Lung and Blood Institute, 2006). The major types of medications which are generally prescribed to lower blood cholesterol particularly LDL-cholesterol include statins, bile acid sequestrants, nicotinic acid, fibrates, and ezetimibe (National Heart Lung and Blood Institute, 2006). Some of the side effects of these drugs include abdominal pain, gas, constipation, liver abnormalities, gout, and high blood sugar (U.S. Food and Drug Administration, 2003; National Heart Lung and Blood Institute, 2006).

Hormone replacement therapy (HRT) has also been an option for postmenopausal women to relieve menopausal symptoms and reduce cholesterol. However, the cardiovascular benefits of HRT have been questioned after the results of the Women's Health Initiative were announced (National Institute of Health, 2002). This study found that women taking estrogen therapy have an increased risk of stroke and blood clots, and women taking estrogen-progestin combined therapy have an increased risk of heart attack, stroke, blood clots, and cancer (National Institute of Health, 2002). Therefore, postmenopausal women are looking for alternative therapies to help lower cholesterol and reduce their CVD risk without the unwanted side effects of HRT and other prescription medications.

Dietary modifications such as increased fiber, phytoestrogens, and antioxidants have previously been shown to reduce cholesterol and CVD risk. (de Kleijn et al., 2002; Lucas et al., 2002; Van Horn et al., 2008). Antioxidants such as anthocyanins, resveratrol, and pterostilbene may play a role in reducing the risk of heart disease (American Dietetic Association, 2005).

Blueberry is an example of a fruit that is a rich source of antioxidants such as anthocyanins, resveratrol, and pterostilbene. The antioxidant capacity of blueberry is among the highest of all fruits and vegetables (Ehlenfeldt and Prior, 2001; Halvorsen et al., 2002; Roy et al., 2002). Previous studies have shown that blueberries may have the ability to prevent heart disease, cancer, and improve neural motor function in the aging (Joseph et al., 1999). However, there are a limited number of studies on the effect of blueberry on lipid parameters, thus the need for this study.

The ***hypothesis*** of this study was that daily consumption of blueberry dose-dependently prevents the ovariectomy-induced rise in blood total cholesterol in a rat model of postmenopausal hypercholesterolemia. To test this hypothesis, we had two ***specific aims***.

1. To determine the extent to which a 2.5%, 5%, and 7.5% (w/w) blueberry diet reduce both liver and serum total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels using the rat model.
2. To determine if blueberries have an effect on serum levels of lipid hydroperoxide and 8-isoprostane, a measure of antioxidative properties.

This study was designed with the assumption that ovariectomized rats will represent postmenopausal women.

Limitations of this study include:

1. The polyphenolic compositions of blueberry were not assessed.
2. The quality of polyphenols were not assessed.

CHAPTER II

REVIEW OF LITERATURE

Cardiovascular disease (CVD) is the leading cause of death worldwide and in the United States. Worldwide, there are approximately 17 million people who die each year from CVD (World Health Organization, 2006) and an estimated 927,000 deaths each year in the United States (Centers for Disease Control and Prevention, 2005; World Health Organization, 2006). The combined direct and indirect costs of major CVD and stroke for 2008 are 448.5 billion dollars (American Heart Association, 2008).

Since 1984, the number of female CVD deaths in the United States has exceeded the number of males with females representing 52.8 percent of CVD deaths (American Heart Association, 2007). In 2004, there were 459,096 females in the United States who died from CVD (American Heart Association, 2007).

Risk Factors for CVD

There are several risk factors for CVD which can be classified into two groups: those which cannot be prevented and those which can be prevented. Risk factors which cannot be prevented include gender, age, and family history. Men over the age of 45 and women over 55 years of age are at increased risk (National Heart Lung and Blood Institute, 2006) Family history is another risk factor which cannot be controlled.

Having a father or brother who was diagnosed with CVD before the age of 55, or a mother or sister diagnosed before the age of 65 can increase an individual's risk of developing CVD.

Other risk factors for developing CVD can often be prevented through lifestyle modifications. This group of risk factors includes being overweight or obese, diabetic, smoking cigarettes, sedentary lifestyle, high blood pressure, or high blood cholesterol.

Obesity is associated with several comorbidities including CVD, type 2 diabetes, hypertension, cancer, and sleep apnea and increases the risk for coronary heart disease (CHD), heart failure, and sudden death through its impact on the cardiovascular system (Poirier et al., 2006). As adipose tissue accumulates, circulating blood volume, plasma volume, and cardiac output must increase causing a dilation of cardiac cavities and increases in wall tension resulting in systemic hypertension, pulmonary hypertension, CHD, or sudden death (Poirier et al., 2006).

Diabetes is associated with an increased risk of several complications including CVD. Nearly 21 million Americans currently have diabetes including 9.7 million women (American Diabetes Association, 2008). CVD accounts for about 65% of all diabetic deaths, and among diabetic women, deaths from CVD have increased 23% over the past 30 years compared to a 27% decrease in women without diabetes (American Diabetes Association, 2008).

Cigarette smoking increases the risk of CVD especially when combined with other risk factors. Smoking causes damage to the lining of the arteries and speeds the progression of atherosclerosis (National Heart Lung and Blood Institute, 2008). Smoking has been shown to increase heart rate and blood pressure and reduce blood flow

(American Heart Association, 2007). Smoking increases the level of carbon monoxide in the blood, depriving the heart and other tissues of needed oxygen (American Heart Association, 2007).

Physical activity can reduce risk of CVD and type 2 diabetes, and can help control weight and blood pressure as well as improving bone density (American Heart Association, 2007; Centers for Disease Control and Prevention, 2007). The American Heart Association recommends that adults engage in a minimum of 30 minutes of moderate intensity activity at least 5 days each week to promote and maintain health (Haskell et al., 2007). However, the number of sedentary Americans continues to increase. In 2000, 38.5% of adults engaged in no leisure time physical activity, and in 2005, this number increased to 40.0% (Centers for Disease Control and Prevention, 2007).

Nearly one in three adults in the US has high blood pressure which if untreated can lead to CVD (American Heart Association, 2007). Blood pressure is measurement of arterial force when the heart beats (systolic pressure) and when the heart is at rest (diastolic pressure). High blood pressure is diagnosed when blood pressure is $\geq$ 140 mm Hg systolic pressure or $\geq$ 90 mmHg diastolic pressure (American Heart Association, 2007). Women who are overweight, have a family history of high blood pressure, or have reached the age of menopause have an increased risk of developing high blood pressure (American Heart Association, 2007). More than 73 percent of women ages 65 to 74 have high blood pressure (American Heart Association, 2007).

High total cholesterol is a major risk factor for developing CVD. When excessive amounts of LDL cholesterol are circulating in the blood, excess amounts are deposited in

the arteries. HDL cholesterol is considered protective because HDL cholesterol has the ability to carry excess cholesterol back to the liver for recycling. The risk of CVD is increased when LDL cholesterol becomes oxidized by free radicals in the arteries (National Heart Lung and Blood Institute, 2006). Oxidized LDL cholesterol accelerates the formation of artery-clogging plaques which stick and accumulate along the lining of the artery walls (American Heart Association, 2007). Free radicals also oxidize polyunsaturated fatty acids in cell membranes, initiating additional changes in artery walls decreasing blood flow.

Total serum cholesterol levels can vary greatly depending on age, sex, heredity, and current health condition; however a serum cholesterol level less than 200 mg/dL is the most desirable. HDL cholesterol of 60 mg/dL or more is considered protective. Having high triglycerides can also place an individual at increased risk of CVD. A desirable range for triglycerides is less than 150 mg/dL (American Heart Association, 2007). A decrease in total cholesterol of just 10% can reduce coronary artery disease risk by 30% (Centers for Disease Control and Prevention, 2005).

Menopause and CVD

In the United States, CVD is the main category of hospitalization discharge for women and is the cause of 39% of all female deaths (American Heart Association, 2007). While CVD risk increases with age for both men and women, the risk drastically increases for women when they reach menopause. On average, menopause occurs when women are between 45 and 55 years of age (World Health Organization, 1996). This increased CVD risk may be related to both metabolic and hormonal changes. After 45

years of age, women are shown to have higher total cholesterol than men of the same age (Ford et al., 2003).

Some CVD risk factors such as hypertension, hypertriglyceridemia, diabetes and low HDL- cholesterol may be stronger risk factors for women than for men (Eaker et al., 1993; Vitale et al., 2007). Menopause is associated with the development of hypertension, central obesity, and dyslipidemia therefore putting postmenopausal women at an increased risk for CVD (de Kleijn et al., 2002). After menopause, there is often an increase in total cholesterol and LDL cholesterol putting women at increased risk for developing CVD (Stevenson et al., 1993; Post et al., 1996; Vitale et al., 2007).

For postmenopausal women, the increased risk of CVD has been attributed to ovarian hormone deficiency (Gorodeski, 2002; Chamberlain et al., 2008). Estrogen deficiency, whether through natural or surgically induced menopause, is an important risk factor for CVD. As a result of decreased estrogen, menopausal transition may act synergistically with gene variations to alter lipoprotein levels (Chamberlain et al., 2008). The Atherosclerosis Risk in Communities (ARIC) study was a longitudinal study of 8,710 women aged 45-64 years that was conducted to determine an association between menopausal status and specific gene polymorphisms, including apolipoprotein E and lipoprotein lipase genes. Postmenopausal women had significantly higher LDL cholesterol, a higher frequency of obesity and overweight, were more commonly diabetic, and were also more likely to be taking cholesterol lowering medication and hormone replacement therapy than premenopausal women (Chamberlain et al., 2008). Chamberlain and colleagues did not find a gene polymorphism which was modified by

menopausal status but determined that the decrease in estrogen may independently affect lipoprotein concentration (Chamberlain et al., 2008).

Post and colleagues (1996) investigated changes in coronary risk factors associated with menopause. They enrolled 610 women from the Framingham Offspring Study who were premenopausal at baseline, not on estrogen replacement, and were 40-57 years at the time of follow-up. These women were classified as premenopausal or postmenopausal if they had undergone natural menopause since the baseline exam eight years previous. Results of the follow-up physical exam indicated that in the postmenopsaul group there was a greater increase in total cholesterol and LDL cholesterol (Post et al., 1996).

In a prospective study investigating the CVD risk of women at the age of 53 years, Kuh and colleagues (2005) enrolled 1303 women aged 53 years and classified them into five groups: premenopausal, perimenopausal, postmenopausal, hysterectomy, or hormone replace therapy user. The purpose of this study was to compare the changes in CVD risk factors such as blood pressure, central and total obesity, total cholesterol, HDL cholesterol, LDL cholesterol, and glycosolated hemoglobin (HbA1c) among groups. Advantages to this study were that the women were all of equal age and previous data had been collected at age 43 years. Results of this study showed that postmenopausal and hysterectomized women had the highest levels of metabolic risk factors (Kuh, et al., 2005).

The relationship between menopause and coronary heart disease (CHD) risk factors were assessed in a cross-sectional study of 1,684 French women aged 45-65 years (Tremollieres et al., 1999). Women were assigned to either a postmenopausal group or

perimenopausal group. All women were given a physical exam including blood samples and then asked to complete a questionnaire relating to CHD risk factors. The postmenopausal group had a significantly higher rate of hypertension and a higher prevalence of obesity and sedentary lifestyle. Elevated serum total cholesterol, LDL cholesterol, and triglycerides were also significantly more frequent in the postmenopausal group (Tremollieres et al., 1999).

A proposed mechanism for the increased CVD risk among postmenopausal women may be related to lipid peroxidation. Postmenopausal women have higher levels of lipid peroxidation than premenopausal women indicating that lipid peroxidation may be the mechanism responsible for the increased CVD risk (Castelao and Gago-Dominguez, 2008).

In summary, menopause puts women at an increased risk of CVD due to an a increase in several CVD risk factors including hypertension, abdominal obesity, and dyslipidemia (Post et al., 1996; Tremollieres et al., 1999; Kuh et al., 2005). After menopause there is increase in total cholesterol and LDL cholesterol putting women at increased risk for developing CVD (Stevenson et al., 1993; Post et al., 1996; Vitale et al., 2007).

Treatment options for lowering cholesterol

Since the 1940s, hormone replacement therapy (HRT) has been shown to be effective in reducing many menopausal symptoms and has also been reported to have positive effects on bone, cholesterol, and CVD risk (Kim et al., 1994). Menopause occurs when ovarian function begins to decline producing less estrogen. With a decline in estrogen, many women begin experiencing adverse menopausal symptoms

and are also at an increased risk for chronic diseases such as CVD and osteoporosis. In the 1940s, the US Food and Drug Administration granted approval of diethylstilbestrol and conjugated equine estrogens to replenish postmenopausal estrogen levels and treat menopausal symptoms (Stefanick, 2005). In the 1970s, progestin was combined with estrogen because women using estrogen alone were found to have an increased risk of developing uterine cancer (American Medical Association, 2005).

In 1991, the National Heart, Lung, and Blood Institute and the National Institute of Health sponsored the Women's Health Initiative (WHI) to investigate the effects of HRT. This was an observational study, a community prevention study, and a two part clinical trial which included a total of 161,000 postmenopausal women (National Institute of Health, 2002). The first part of the clinical trial included 16,608 women taking estrogen-progestin combined therapy or a placebo. The second part included 10,739 women taking estrogen-alone therapy or a placebo. Both components of the clinical trial were stopped early when researchers found that the risks to participants outweighed the benefits of treatment. Estrogen-alone therapy put women at an increased risk of stroke and blood clots. Women taking estrogen-progestin combined therapy were not only put at an increased risk of stroke and blood clots, but were also at an increased risk of developing heart disease and breast cancer.

In 2003, the results of a sub-study of the WHI, the Women's Health Initiative Memory Study (WHIMS), announced its findings that older women taking combination hormone therapy have twice the risk of developing dementia, including Alzheimer's disease, compared to women taking the placebo (National Institute of

Aging, 2003). Then, in 2004, investigators of the WHIMS announced further findings that estrogen-alone hormone therapy could also increase an older woman's risk for developing dementia (National Institute of Health and Aging, 2004). Researchers found that women taking estrogen-alone therapy had a 49% greater risk for developing dementia than the group taking the placebo.

To determine if the results of the Women's Health Initiative had an effect on the current use of hormone replacement therapy, Kelly et al. (2005) conducted a survey of 3853 women who were 50 years of age and older. Participants in this study were interviewed to estimate current medication use. The findings from this survey indicated that there is a large decline in the use of hormone therapy by postmenopausal women after the results of the WHI were announced (Kelly et al., 2005).

There are medications that can be prescribed to help improve cholesterol levels which include statins, bile acid sequestrants, nicotinic acid, fibrates, and ezetimibe (National Heart Lung and Blood Institute, 2006; American Heart Association, 2007). Statin drugs include: atorvastatin (Lipitor), fluvastatin (Lescol), lovastatin (Mevacor), pravastatin (Pravachol), simvastatin (Zocor), and rosuvastatin (Crestor). Statins lower cholesterol by blocking the action of the liver enzyme HMG Co-A reductase thus reducing the synthesis of cholesterol (National Heart Lung and Blood Institute, 2006). Statins can also offer antiatherosclerotic effects beyond improvement of plasma lipids. These benefits include stabilizing plaques, improving vascular relaxation, and promoting new vessel formation (Ikeda and Shimada, 2001). Statins may also inhibit both prepro-

endothelin (ET-1) contraction and DNA synthesis in vascular smooth muscle (Mraiche et al., 2005).

Although statins can be effective at improving cholesterol, they can produce side effects such as abdominal pain, gas, and constipation (American Heart Association, 2008). Individuals taking statins can also develop liver and muscle problems (National Heart Lung and Blood Institute, 2006). Statins may also produce adverse effects to the oral cavity. A recent study by Cruz and colleagues (2008) found that statin use was associated with oral symptoms such as dryness and itching of the tongue and lips and increased coughing (Cruz et al., 2008).

Bile acid sequestrants exert lipid lowering effects by binding to bile acids and promoting their excretion in the feces causing the liver to use cholesterol for the synthesis of new bile acids. This effectively reduces LDL-cholesterol levels. Bile acid sequestrants include: colestipol (Colestid), colesevelam (Welchol), and cholestyramine (Questran). Bile acid sequestrants can also be effective in lowering LDL-cholesterol by 10 to 20 percent; however these drugs have been shown to cause gas, bloating, and constipation (National Heart Lung and Blood Institute, 2006). Bile acid sequestrants can also raise triglyceride levels (American Heart Association, 2008).

Nicotinic acid or niacin is a water soluble B vitamin which improves lipoprotein levels. Nicotinic acid is thought to improve lipid levels by decreasing fatty acid mobilization and inhibiting synthesis of triglycerides which leads to increased intracellular apo B degradation and reduced secretion of VLDL and LDL particles (Ganji et al., 2003). Nicotinic acid can lower LDL cholesterol by 10 to 20 percent and reduce triglycerides by 20 to 50 percent (National Heart Lung and Blood Institute, 2006) and can

also increase HDL cholesterol (Birjmohun et al., 2005). Nicotinic acid is available in three forms: immediate release, timed release, and extended release (National Heart Lung and Blood Institute, 2006). Although nicotinic acid can help lower cholesterol levels, individuals often experience bothersome side effects such as flushing or hot flashes which occur from dilation of the blood vessels (National Heart Lung and Blood Institute, 2006). Nicotinic acid can also cause a variety of gastrointestinal side effects such as nausea, indigestion, gas, vomiting, and diarrhea, and individuals must be closely monitored due to a variety of serious side effects such as liver abnormalities, gout, and high blood sugar (National Heart Lung and Blood Institute, 2006).

Fibrates are a class of cholesterol lowering drugs that are primarily effective at reducing triglycerides and may increase HDL cholesterol (Birjmohun et al., 2005). The effects of fibrates on lipid metabolism are mostly mediated through the activation of peroxisome proliferator-activated receptors (PPARα) improving the plasma transport rate of lipoproteins (Watts and Dimmitt, 1999). Individuals taking fibrates generally experience a 20 to 50 percent reduction in triglycerides and an increase of 10 to 15 percent in HDL cholesterol (National Heart Lung and Blood Institute, 2006). However, individuals taking fibrates are put at increased risk of developing cholesterol gallstones, and fibrates can interfere with other medications (National Heart Lung and Blood Institute, 2006).

Ezetimibe can also be effective at controlling cholesterol by blocking the absorption of biliary and dietary cholesterol within the intestinal tract. Ezetimibe localizes at the brush border of the small intestine and decreases cholesterol uptake into the enterocytes (Nutescu and Shapiro, 2003). Ezetimibe can reduce LDL cholesterol by

15 to 20 percent (Nutescu and Shapiro, 2003) and co-administering with a statin can increase its effectiveness (Lipka, 2003). However, ezetimibe can cause side effects such as abdominal pain and fatigue (U.S. Food and Drug Administration, 2003).

Although cholesterol lowering medications may be effective, these medications do not come without unwanted side effects. They are also expensive and often require lifelong treatment (Hu, 2005).

Dietary Modifications

Because of the risks and unwanted side effects associated with hormone therapy and other medications, postmenopausal women are looking for alternative therapies to help lower cholesterol and reduce their CVD risk. There have been many studies conducted which have shown that dietary modifications such as increased fiber intake, phytoestrogens, antioxidants, and polyphenols such as anthocyanins, resveratrol, and pterostilbene may improve cholesterol and reduce CVD risk (American Dietetic Association, 2005; American Heart Association, 2007).

Fiber

An increased intake of dietary fiber may help prevent CVD (Marlett et al., 2002; American Heart Association, 2007). The adult treatment panel III recommends viscous (soluble) fiber as a therapeutic dietary option to enhance lowering of LDL cholesterol (National Cholesterol Education Program. Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults, 2002). Fiber is an indigestible carbohydrate and is present in plant foods such as grains, vegetables, and fruits. Fiber is

classified into two groups, soluble fiber and insoluble fiber. Soluble fiber, or viscous fiber, is found in foods such as oats, beans, peas, seeds, barley, citrus fruits, strawberries, blueberries, and apple pulp. Insoluble fiber is found in foods such as whole-wheat breads, cereals, rye, rice, barley, vegetables, and apple skin.

Consumption of dietary fibers, especially viscous fibers, has been associated with lower blood cholesterol levels and improved blood glucose and insulin levels (Marlett et al., 2002; Galisteo et al., 2008). These benefits show that fiber consumption may help prevent CVD and diabetes. To help reduce cholesterol, the American Heart Association recommends eating foods that contain both soluble and insoluble fiber (American Heart Association, 2007). Current recommendations for dietary fiber consumption are 21-38 grams/day (American Dietetic Association, 2006).

The cholesterol lowering benefits of fiber may be related to its viscosity and fermentability (Marlett et al., 2002). As food is digested, fiber interferes with bile acid absorption and bile is excreted from the body. LDL cholesterol is then removed from the blood and new bile acid is synthesized to replace bile acids lost in the stool (Marlett et al., 2002).

High fiber intake has been linked to reduced cholesterol and CVD risk in several studies (Jenkins et al., 2003; Galisteo et al., 2008). A study by Queenan et al, (2007) randomly assigned 75 hypercholesterolemic males and females to receive either 6 grams per day of concentrated oat-glucan, a fermentable fiber or dextrose as control. After six weeks, researchers found that the treatment group had a significant reduction in total and LDL cholesterol (Queenan et al., 2007).

The efficacy of a dietary portfolio including viscous fibers was compared to statin treatment in a randomized parallel study of 46 hyperlipidemic adults (Jenkins et al., 2003). Participants were assigned to a low fat diet (control), low fat diet plus 20 mg lovastatin (statin group), or a low fat diet including plant sterols, viscous fibers, soy protein and nuts (dietary portfolio group). After 4 wks of treatment, LDL cholesterol concentrations decreased by 8.5%, 33.3%, and 29.6% in control, statin, and dietary portfolio group, respectively. There was no significant difference in cholesterol reduction between the dietary portfolio group and the statin group. These results indicated that dietary modifications which include viscous fibers and vegetable proteins such as soy and nuts may have a similar ability to reduce LDL cholesterol to treatment of a statin drug (Jenkins et al., 2003).

A fiber rich diet may also help reduce blood pressure in adults with hypercholesterolemia (Behall et al., 2006). Insoluble fiber (whole wheat and brown rice) and soluble fiber (barley) were found to reduce diastolic and mean arterial blood pressure in hypercholesterolemic adults (Behall et al., 2006).

The effects of soluble fiber intake on CVD risk was assessed in a study of 9776 adults who participated in the National Health and Nutrition Examination Survey I (NHANES I) Epidemiologic Follow-up Study. Baseline data from NHANES I was collected between 1971 and 1975 and included dietary assessments, medical examination, and laboratory tests. Follow-up data was collected 19 years later and revealed dietary fiber, particularly water-soluble fiber, was significantly associated with CVD incidence and mortality (Bazzano et al., 2003). Individuals consuming

more than 4 grams of soluble fiber per 1735 kcals have a 15% reduced risk of CHD, 10% reduced risk of CVD, and a 12% lower CVD mortality (Bazzano et al., 2003).

The effects of fiber on metabolic syndrome have been investigated (Galisteo et al., 2008). Metabolic syndrome is the term to describe a cluster of abnormalities which increase an individual's risk of CVD. Metabolic syndrome includes central obesity, low concentrations of plasma HDL cholesterol, high levels of triglycerides, hypertension, hyperglycemia, and insulin resistance (Galisteo et al., 2008). Dietary fiber was found to improve all metabolic syndrome abnormalities including improved lipids, weight, and insulin sensitivity (Galisteo et al., 2008).

In a recent study, dietary fiber from cocoa was found to help reduce lipids in hypercholesterolemic rats (Lecumberri et al., 2007). Researchers in this study tested the cholesterol lowing effects of 165g cocoa fiber /kg diet of which 80% was soluble fiber. Rats which were fed a diet containing cocoa fiber had a significant reduction in LDL cholesterol, total serum cholesterol, and triglycerides.

Antioxidants

Oxidative stress is linked to the development of hypertension, hyperglycemia, and hyperlipidemia (Kaliora and Dedoussis, 2007). Oxidative stress induced by reactive oxygen species (ROS) initiates plaque formation through the oxidation of the LDL cholesterol (Rao, 2002). Macrophages inside the arterial wall take up the oxidized LDL and cause the formation of plaque (Rao, 2002). This plaque formation accumulates and sticks to arterial lining causing atherosclerosis. Consuming foods high in antioxidants may lower CVD risk by preventing the oxidation of cholesterol.

In a double-blind and placebo-controlled study, 2002 patients with coronary atherosclerosis were enrolled to investigate the link between the antioxidant vitamin E (α-tocopherol) and CVD progression (Stephens et al., 1996). Individuals were randomized to one of three groups: 800 IU/ day α-tocopherol capsules, 400 IU/ day α-tocopherol capsules, or placebo capsules. Plasma α-tocopherol concentrations increased in the both treatment groups but did not change in the placebo group. α-tocopherol treatment significantly reduced the risk of the non-fatal myocardial infarction (Stephens et al., 1996).

Devaraj and colleagues (2007) investigated the effects of a high dose vitamin E supplement (1200 IU/day for 2 years) on biomarkers of oxidative stress and inflammation in a double blind, randomized, placebo controlled trial. The vitamin E form RRR- α-tocopherol was used in this study because it has been shown to exhibit both antioxidant and anti-inflammatory activity (Devaraj et al., 2007). There were no changes in the lipid profile of treatment groups compared to placebo, however plasma α-tocopherol levels were significantly higher in the group receiving RRR- α-tocopherol supplementation, and supplementation significantly reduced LDL oxidation (Devaraj et al., 2007).

In a study evaluating the beneficial effect of tomatoes, a rich source of the antioxidant, lycopene on coronary heart disease (CHD), Bose and Agrawal (2007) investigated the lipid peroxidation rate of serum enzymes such as superoxide dismutase, glutathione peroxidase, and glutathione reductase, as well as the lipid profile of participants with CHD or an age-matched control group. After 60 days of tomato supplementation, participants showed a significant improvement of serum

enzyme levels and decreased lipid peroxidation rate compared to the control group. However, there were no significant changes in lipid profile (Bose and Agrawal, 2007).

The best way to add antioxidants to the diet is to consume generous servings of fruits and vegetables daily. Adebawo and colleagues (2006) studied the effects of a diet rich in fruits and vegetables on CVD risk factors. These researchers enrolled 20 African individuals with hypertension and looked at the effects of consuming a diet rich in locally grown fruits and vegetables on CVD risk factors. They found that eating fruits and vegetables was associated with decreases in serum triglycerides, total cholesterol, and LDL cholesterol, thus reducing CVD risk factors.

Phtyoestrogens

Phytoestrogens are naturally occurring plant compounds with similar structures and function to that of estradiol. Phytoestrogens have been found to promote health and may help reduce CVD risk and offer a variety of postmenopausal benefits (Lissin and Cooke, 2000). Studies on phytoestrogens have reported decreases in postmenopausal symptoms and a decreased incidence of osteoporosis and breast cancer (Setchell and Cassidy, 1999; Lissin and Cooke, 2000). The three most common phytoestrogens and their sources include isoflavones (soybeans), lignans (flaxseed), and coumestans (alfalfa sprouts) (Lissin and Cooke, 2000).

A cross-sectional study of 939 postmenopausal women investigated the association between the intake of isoflavones and lignans and CVD risk factors such as blood pressure, waist-hip ratio, and plasma lipoprotein levels (de Kleijn et al., 2002). CVD risk factors such as waist-hip ratio and plasma triglyceride levels were

lower in women consuming more isoflavones and lignans (de Kleijn et al., 2002). The results of this study indicate that phytoestrogens may have the potential to reduce CVD risk factors in postmenopausal women (de Kleijn et al., 2002).

To determine the effects of soy foods on markers for CVD and osteoporosis, forty-two postmenopausal women were asked to consume three daily servings of whole soy foods containing approximately 60 mg/d of isoflavones for 12 weeks (Scheiber et al., 2001). Results of this study indicate that 60 mg/d of isoflavones may result in reductions in several key clinical risk factors for CVD and osteoporosis in postmenopausal women (Scheiber et al., 2001). Lucas et al. (2003) demonstrated that soy isoflavones could help reduce CVD risk factors and prevent hypercholesterolemia in a hamster model of postmenopausal hypercholesterolemia. These studies show that dietary modifications which include phytoestrogens may help reduce CVD risk factors.

Polyphenols

Polyphenols are a subclass of antioxidants which are present in many fruits and vegetables. Polyphenols such as anthocyanins, resveratrol, and pterostilbene are abundant in berries, grapes, and wine and have been studied to determine their relationship to CVD and other chronic diseases.

Anthocyanins are antioxidant compounds which make up the bright blue, violet, red, and purple colors of fruits and vegetables. Anthocyanins are particularly rich in soft fruits such as red grapes, blueberries, raspberries, and cranberries. The cardioprotective benefits of grape polyphenols were investigated in a single-blind,

crossover study design of 24 pre- and 20 post-menopausal women (Zern et al., 2005). Subjects were asked to consume 36 g/day grape powder or placebo for 4 weeks followed by a 3 week washout period, then alternate treatments. They found that the grape powder significantly reduced plasma triglycerides, LDL cholesterol, and urinary isoprostane concentrations.

Resveratrol is found in grapes, berries, and peanuts. It is the heart healthy antioxidant found in red wine and may reduce the risk of heart disease and cancer (American Dietetic Association, 2005). Curtis and colleagues (2005) investigated the effects of alcohol free red wine on arterial thrombosis in order to determine if the beneficial effects of red wine were related to wine consumption or the alcohol. They found that the benefits from the red wine were related to polyphenols in the wine and not the alcohol (De Curtis et al., 2005).

Pterostilbene has been shown to have lipid lowering effects in hamsters (Rimando et al., 2005). The researchers first investigated the effects of resveratrol and the analogues pterostilbene, piceatannol, and resveratrol trimethyl ether on the peroxisome proliferator-activated receptor α- isoform (PPARα). PPARα plays a role in fatty acid and lipid metabolism and can increase the β-oxidation of fatty acids and cause a reduction in triglycerides and VLDL cholesterol. PPARα can also lead to an increase in HDL cholesterol by causing an induction of hepatic apolipoprotein A-I and A-II expression. Results of their analysis indicated that of the four stilbene analogues, pterostilbene had the highest effect on PPARα , and its results were higher than cirofibrate, a hypolipidemic drug. The researchers then expanded their study and randomly assigned eighteen 7-8 week old male golden Syrian hamsters to receive a high fat control diet or

high fat diet fortified with pterostilbene for 21 days. Results of this study indicated that the pterostilbene fed hamsters had 29% lower LDL cholesterol and 7% higher HDL cholesterol than the control group (Rimando et al., 2005).

Blueberries

The antioxidant capacity of blueberries is among the highest of fruits and vegetables (Ehlenfeldt and Prior, 2001; Halvorsen et al., 2002; Roy et al., 2002). Blueberries contain anthocyanins, resveratrol, and pterostilbene. Although there have been many studies which have shown cardiovascular benefits from eating a diet rich in fruits and vegetables, there have been a limited number of studies showing the relation of blueberries to CVD. However, there have been many recent studies which have shown benefits of blueberry consumption and improved brain function (Andres-Lacueva et al., 2005; Sweeney et al., 2002).

Joseph et al. (1999) conducted a study to determine if fruits with high antioxidant activity, including blueberry, could reverse age-related declines in neural and behavioral functions. These researchers used forty 19-month old male rats which were assigned to one of four treatment groups: control, 1.48% strawberry, 0.91% spinach, or 1.86% blueberry. The amounts of fruits were based on ORAC activity so each diet provided equivalent antioxidants. Rats were fed their assigned diet for 8 weeks before neural and psychomotor behavior testing. Researchers found that these fruit extracts were able to prevent age-related neuronal and behavioral dysfunctions with blueberry supplementation showing the greatest effect on reversing the deleterious effects of aging on calcium

homeostasis. The blueberry fed rats were also the only group to show reversals in motor behavioral deficits (Joseph et al., 1999).

In a study looking at the effects of blueberry on memory, researchers examined the effects of blueberry consumption using a rat model to determine if the polyphenols from a 2% blueberry supplemented diet could be found in brain areas corresponding with cognitive performance. The researchers found that several anthocyanins were found in the cerebellum, cortex, hippocampus or striatum of the rats receiving the blueberry diet. The findings from this study suggest that there is a relationship between learning and memory in rats fed a blueberry supplemented diet (Andres-Lacueva et al., 2005).

Sweeny and colleagues (2002) investigated the effects of blueberry on ischemia-induced brain damage. Researchers fed a 14.3% blueberry diet to male Long-Evans rats for six weeks before ligation of the left common carotid artery followed by hypoxia to induce stroke. One week after the simulated stroke, researchers determined that rats on the blueberry diet had only a 17 ± 2% loss of neurons to the ischemic hippocampus compared to a loss of 40 ± 2% in the control group. This study shows that the consumption of blueberries may help protect against ischemia-induced brain damage, and may help improve stroke outcomes (Sweeney et al., 2002).

While these previously mentioned studies were looking at benefits from blueberry other than cardiovascular benefits, these studies were able to show the potential of blueberry to help protect against chronic degenerative diseases. Consuming a diet rich in antioxidants has been recommended as a way to help prevent chronic diseases such as CVD. Eating a diet rich in blueberries has been found to increase serum antioxidant

status, and therefore may help prevent the development of cardiovascular disease (Kay and Holub, 2002).

CHAPTER III

METHODOLOGY

Animals and Treatment Groups

Sixty two 5-month old Sprague-Dawley rats (Harlan Sprague-Dawley Inc., Indianapolis, IN) were housed two per cage in an environmentally controlled laboratory at the Laboratory for Animal Research at Oklahoma State University, Stillwater, OK. Guidelines for the ethical care and treatment of animals from the Animal Care and use Committee at Oklahoma State University were strictly followed. The rats were acclimated for three days and were either sham-operated (SHAM; 1 group) or ovariectomized (OVX; 4 groups) with 12-13 rats per group. After surgery, rats were assigned to one of four dietary treatments for ninety days: control diet (SHAM and one OVX group), 2.5% blueberry diet (OVX + 2.5%), 5% blueberry diet (OVX + 5%), or 7.5% blueberry diet (OVX + 7.5%). Experimental groups are shown in Figure 1. The compositions of the experimental diets are shown in Table 1. Diet was restricted to the mean food intake of the SHAM control group and all rats had free access to deionized water. Food intake was monitored every three days and body weight was monitored weekly.

Animal Necropsy and Processing of Tissue Samples

At the end of the ninety day treatment period, rats were fasted for 12 hours and placed in metabolic cages. The rats were then anesthetized with a mixture of ketamine hydrochloride (100 mg/kg body weight) and xylazine (5 mg/kg body weight) and bled from the abdominal aorta. Blood samples were collected and centrifuged using the Jouan CR3i tabletop centrifuge (Winchester, VA). Samples were centrifuge at 4000 rpm for 20 minutes at 4°C to separate serum. Aliquots of serum were kept frozen at -80° C until analyzed.

The liver was immediately removed, rinsed with ice-cold saline solution, and total liver weight was recorded. The liver was then placed in a sealed container and kept frozen at -80°C until analysis. The uterus and spleen were removed, weighed, and discarded.

Serum parameters

Serum total cholesterol, HDL cholesterol, and triglycerides were measured using the Alfa Wassermann clinical chemistry analyzer (West Cadwell, NJ). Other clinical parameters such as albumin (ALB), alkaline phosphatase (ALP), blood urea nitrogen (BUN), calcium, creatinine, magnesium, phosphorus, and total protein were also analyzed using the Alfa Wassermann clinical chemistry analyzer (West Cadwell, NJ). The analyzer was calibrated using Gemcal reference serum (Alfa Wassermann; West Caldwell, NJ). Lipid concentrations were determined using commercially available kits and checked with a high and low quality control (Alfa Wasserman; West Caldwell, NJ). The tests were performed according to the manufacturer's instructions.

Serum total cholesterol was determined using a reagent containing cholesterol esterase. The cholesterol esterase is used to release cholesterol from its esters producing hydrogen peroxide. When combined with 4-aminoantipyrine (AAP) and p-hydroxybenzoic acid, a red color is produced which is directly proportional to cholesterol concentration and measured photometrically at 505 nm.

HDL cholesterol was measured using a detergent which solubilizes HDL lipoprotein particles allowing HDL cholesterol to react with cholesterol esterase and cholesterol oxidase. In the presence of a chromogen, a color is produced which is directly proportional to HDL cholesterol concentration and can be determined by measuring the increase in absorbance bichromatically at 592/692 nm.

Triglycerides were measured using a reagent which causes a series of enzymatic reactions resulting in the production of H_2O_2. The H_2O_2 reacts with the p-chlorophenol and AAP catalyzed by peroxidase producing a red-colored complex which is directly proportional to triglyceride concentration and can be measured at an absorbance of 505 nm.

Liver lipid parameters

Total liver lipids were determined using the Folch gravimetric method (Folch et al., 1957). Approximately two grams of liver samples were homogenized, placed in 50 ml centrifuge tubes, and extracted with 25 ml chloroform: methanol (2:1u/u) mixture. Sodium chloride solution (0.73%) was added to the chloroform: methanol mixture. Tubes were vortexed and left standing until two phases were distinctly separated. The top aqueous layer was aspirated, and the bottom organic layer was transferred to a 25 ml

volumetric flask and diluted to the mark with chloroform: methanol (2:1). An aliquot of 2 ml of the organic layer was used for cholesterol analysis and the remainder of the solution was poured into pre-dried and pre-weighed aluminum pans for total lipid analysis.

The filled aluminum pans were placed under a fume hood overnight to allow the solvent to evaporate. The pans were then placed in a 100°C oven for 1 hour and cooled in a dessicator for 30 minutes. The pans were then weighed and recorded to determine total lipid content.

A 2 ml aliquot of lipid extracted by the Folch method was used to determine liver total cholesterol according to the color reaction method by Searcy and Bergquist (1960). The lipid extract was placed in duplicate into 25 x 125 mm culture tubes and evaporated under nitrogen gas. Once dried, 15 ml saponification solution (15% alcoholic KOH solution and 3% pyrogallic acid in 90% ethanol) was added to all tubes. Tubes were then placed in a shaking water bath for 10 minutes at 88°C. After tubes were cooled, 5 ml distilled water and 10 ml hexane were added to the tubes. Tubes were vortexed and left standing to separate. An aliquot of 5 ml supernatant was pipetted into another 25 x 125 mm tube and placed under the fume hood to evaporate. Once tubes were dry, 400 µl acetone: ethanol (1:1), 6 ml saturated $FeSO_4$-$7H_2O$ in glacial acetic acid and 2 ml concentrated sulfuric acid were added to each tube. Tubes were vortexed, cooled, and absorbance was read at 490 nm.

Lipid hydroperoxide

Lipid hydroperoxide (LPO) was assessed using a commercially available kit from Cayman Chemical (Ann Arbor, MI) to measure the hydroperoxides utilizing reduction/oxidation reactions with ferrous ions. The assay was performed using an extraction method with chloroform to prevent an overestimation of LPO. Serum samples were aliquoted into glass test tubes and prepared according to manufacturer's instructions. An equal amount of 600µl Extract R (crystalline solid saturated in ethanol) was added to each sample and vortexed. One ml of cold chloroform was then added to each tube, vortexed, and centrifuged at 1,500 x g for 5 minutes. The bottom chloroform layer was pipetted, transferred to another test tube, and stored on ice. An aliquot of chloroform-methanol solvent (1:1, v/v) was added to 500 µl of each sample followed by 50 µl of chromogen. Three hundred µl of samples were pipetted into a 96-well plate and absorbance at 500 nm was measured.

8- Isoprostane

8-isoprostane was determined using the competitive enzyme immunoassay (Cayman Chemical, Ann Harbor, MI). Because 8-isoprostane and 8-isoprostane-acetylcholinesterase (AChE) compete for 8-isoprostane-specific rabbit antiserum binding sites, this assay measures the amount of 8-isoprostane-acetylcholinesterase (AChE) or 8-isoprostane tracer that is able to bind to rabbit antiserum as this is inversely proportional to the concentration of 8-isoprostane of the sample. Fifty µl of serum or standards were pipetted into the wells of a 96-well plate which was supplied with the assay kit. Samples were assayed in duplicate and 50 µl of 8-isoprostane AChE tracer and 50 µl 8-

isoprostane antiserum were added to each sample well. The plate was incubated for 18 hours at room temperature. Wells were then emptied and washed five times with wash buffer. Ellman's Reagent was prepared and added to each well. The plate was covered and placed on an orbital shaker for 60 minutes to develop in the dark before being read at 420 nm.

CHAPTER IV

RESULTS

Food intake, body and tissue weights

Food intake, body and tissue weights are presented in Table 2. Food intake among treatment groups averaged approximately 14 g per day. There was no significant difference in food intake between groups as rats were pair-fed to the mean food intake of the sham group. Because rats were randomly assigned to treatment groups based on body weight, there was no significant difference in initial body weight. However, after 90 days of treatment, the weight of the sham control group was significantly lower than all the OVX groups despite pair feedings. There was a significant reduction in uterine weight in the OVX groups indicating success of ovariectomy. Blueberry did not induce an increase in uterine weight. There was no significant difference in the weight of liver or spleen among treatment groups.

Lipid parameters

As we have observed in previous studies (Lucas et al., 2003; Lucas et al., 2004), ovariectomy significantly increased serum total and non-HDL cholesterol concentrations (Figure 2). Ovariectomy caused a 35% and 31% increase in total and non-HDL

cholesterol, respectively. The doses of blueberry used in the study were not effective at reducing the rise in cholesterol due to ovariectomy. HDL cholesterol was increased due to ovariectomy and blueberry had no effect (Table 3). There was no significant difference in serum triglycerides, liver total lipids, and liver cholesterol among groups (Table 3).

Serum parameters

Results of other serum clinical parameters are presented in Table 4. Alkaline phosphatase (P=0.0041) and glucose (P=0.0129) levels increased significantly with ovariectomy and blueberry has no effect on these parameters. Serum phosphorus (P=0.0004) and blood urea nitrogen (P=0.0054) levels showed significant decreases with ovariectomy, with BB having no significant effect on these parameters. Serum albumin (P<0.0001) and total protein (P=0.0001) decreased significantly with ovariectomy. Among the OVX groups, these clinical parameters were highest with the 5% BB diet. The serum levels of calcium and creatinine did not differ among groups.

Lipid hydroperoxide

Ovariectomy has no effect on serum levels of lipid hydroperoxide (Figure 3). Unexpectedly, blueberry consumption tended to increase serum lipid hydroperoxide.

8-Isoprostane

Ovariectomy did not cause significant changes in serum isoprostane levels (Figure 4). The 8-isoprostane levels appeared to decrease with higher concentrations of blueberry, but there were no significant differences between groups.

CHAPTER V

DISCUSSION

Serum cholesterol is a major risk factor for the development of CVD. This study investigated whether blueberry was effective at preventing an increase in serum total cholesterol in a rat model of postmenopausal hypercholesterolemia. Blueberry was chosen for this study due to its rich antioxidant content. The total antioxidant capacity of blueberry is among the highest of all fruits and vegetables (Ehlenfeldt and Prior, 2001; Halvorsen et al., 2002; Roy et al., 2002). Antioxidants are associated with a decreased risk of CVD by preventing the oxidation of cholesterol. Blueberries have been found to increase serum antioxidant status, and therefore may help prevent the development of cardiovascular disease (Kay and Holub, 2002).

Blueberries also contain polyphenols such as anthocyanins, resveratrol, and pterostilbene. An increased intake of polyphenols may improve cholesterol and reduce CVD risk (American Dietetic Association, 2005; American Heart Association, 2007). Previous studies have shown that blueberries may have the ability to prevent heart disease, cancer, and improve neural motor function in the aging (Joseph et al., 1999).

Blueberry is also a source of soluble fiber which is associated with lower cholesterol and improved blood glucose and insulin levels (Marlett et al., 2002; Galisteo et al., 2008) and has also been found to improve other metabolic syndrome abnormalities

including hypertension (Behall et al., 2006), weight control, and improved insulin sensitivity (Galisteo et al., 2008).

Unfortunately, the results of this study did not find blueberries to have cholesterol lowering properties. However, other studies looking at oxidative biomarkers have found cardiac benefits without improvements to the lipid profile. Devaraj and colleagues (2007) found that supplementation with the antioxidant vitamin E did not alter lipid profile but did significantly reduce LDL oxidation (Devaraj et al., 2007). In another study, participants consuming antioxidant rich tomatoes showed a significant decrease in lipid peroxidation rate but there were no significant changes in lipid profile (Bose and Agrawal, 2007). Therefore, we determined that oxidative biomarkers should be investigated in this study due to blueberry's high antioxidant content. However, in this study, blueberry consumption unexpectedly tended to increase serum lipid hydroperoxide and had no significant effect on 8-isoprostane levels. These findings were surprising considering blueberry's high antioxidant capacity.

A possible reason why this study had unexpected findings is that the quantity and quality of polyphenols were not measured prior to this study. Although blueberries are recognized for their high antioxidant and phenolic content, we are uncertain of the phenolic content of the blueberries used in this study.

In this study, an ovariectomized rat model was used. Although this animal model is economical and well suited for a joint study on bone loss, the golden Syrian hamster may be a better model. Ovariectomized hamsters experience changes in lipids comparable to postmenopausal women which make them an appropriate model for

studying cholesterol metabolism (Sohn et al., 1999).

It would be interesting to see if this study would have had different results if a greater amount of blueberry had been used. In this study, treatment groups consisted of 2.5%, 5%, and 7.5% blueberry. However, other studies involving blueberry have found benefits with greater doses. In a study which found that blueberries can alter the composition and structure of the rat aorta, treatment consisted of an 8.0% blueberry enriched diet (Kalea et al., 2006). Additionally, in a study investigating the effects of blueberry on ischemia-induced brain damage, Sweeny and colleagues (2002) determined that the consumption of a 14.3% blueberry diet may help protect against ischemia-induced brain damage, and may help improve stroke outcomes.

Although the above studies did not investigate the effects of blueberry on lipid parameters, studies with similar dietary modifications have shown lipid profile improvements with higher treatment doses than this current study. Experimental diets of 12% strawberry and plum powder have resulted in a significant decrease in serum and liver cholesterol in a rat model (Mateos et al., 2005). Cocoa powder, which is a rich source of polyphenols and fiber, has resulted in significant cholesterol reductions at both 12% (Mateos et al., 2005) and 16.5% (Lecumberri et al., 2007).

In summary, dietary modifications play an important role in reducing cholesterol and other CVD risk factors. Diets rich in antioxidants from fruits and vegetables have been associated with a decreased risk of CVD. Soluble fiber and phytoestrogens have also been vastly studied for their potential to reduce cholesterol and CVD risk. The need for dietary alternatives is especially important for postmenopausal women considering the side effects of HRT and cholesterol lowering medications. Although this current

study did not find improvements to the lipid profile of ovariectomized rats, there is a need for further studies of blueberries due to their rich antioxidant and phenolic concentrations.

Figure 1: Experimental Design

Sham: Sham operated; OVX: Ovariectomized. Rats were fed assigned treatment diet for 90 days.

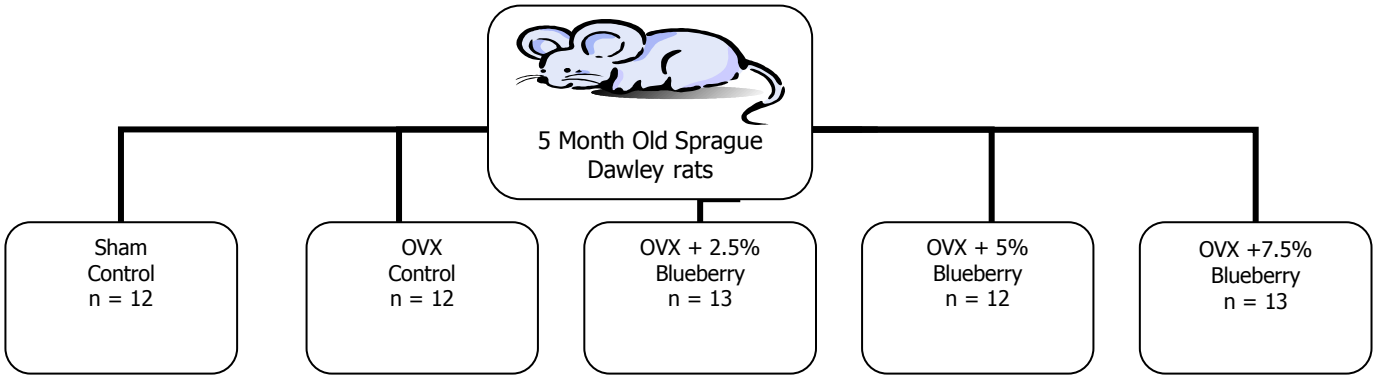
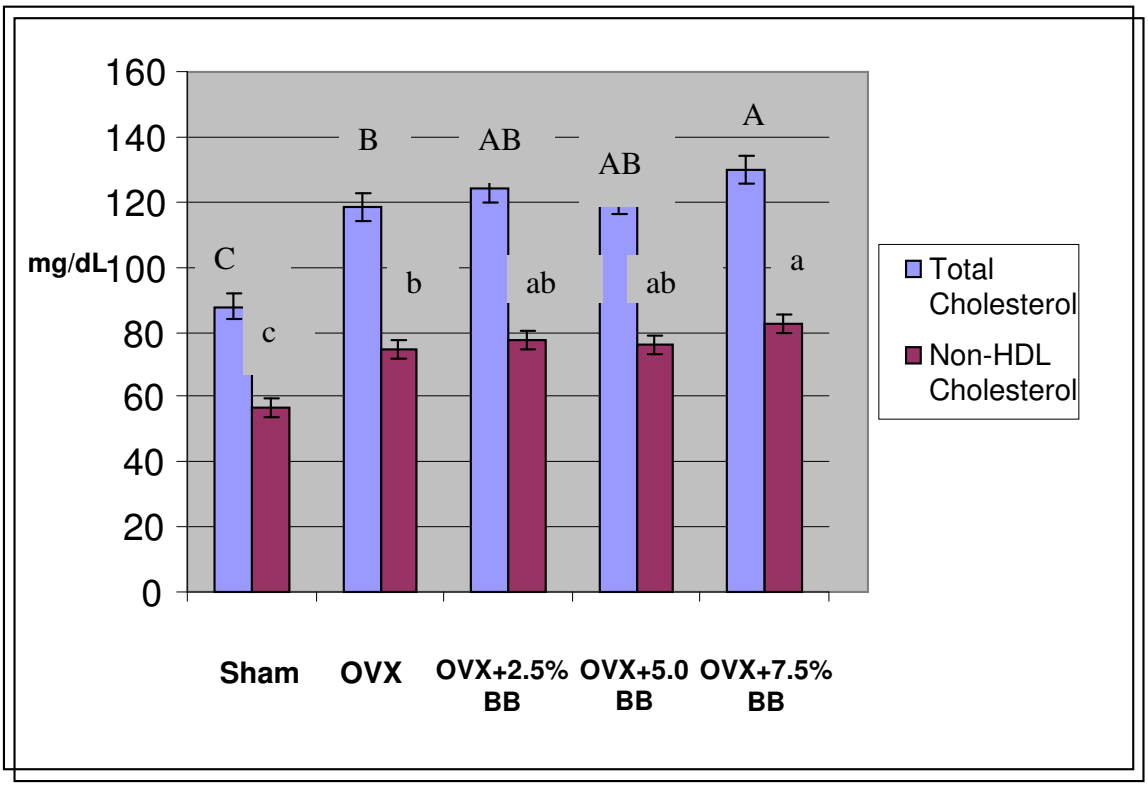
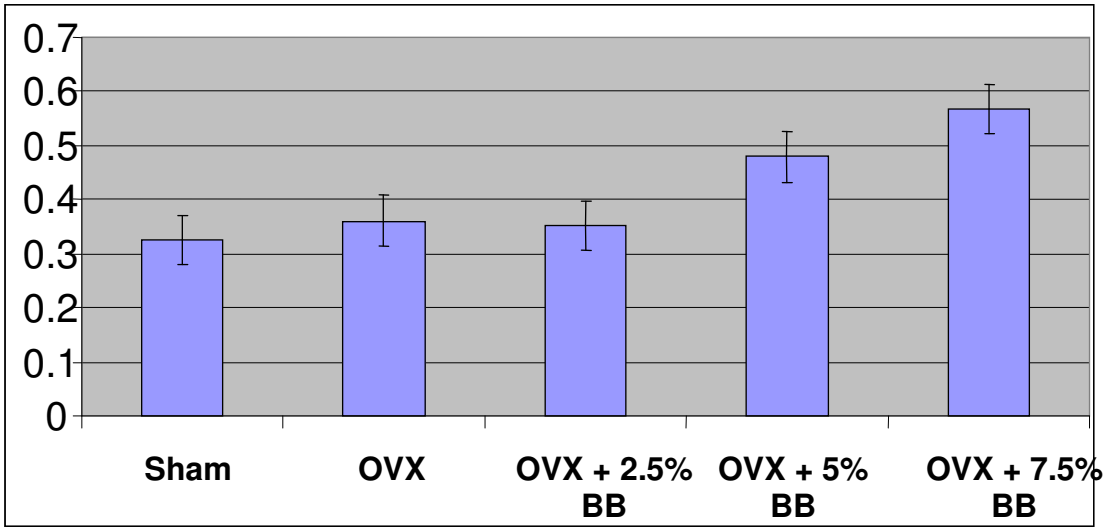


Figure 2: The effects of the 90 day intake of three doses of blueberry (BB) on serum total cholesterol and non- HDL cholesterol of ovariectomized (OVX) rats [1,2]



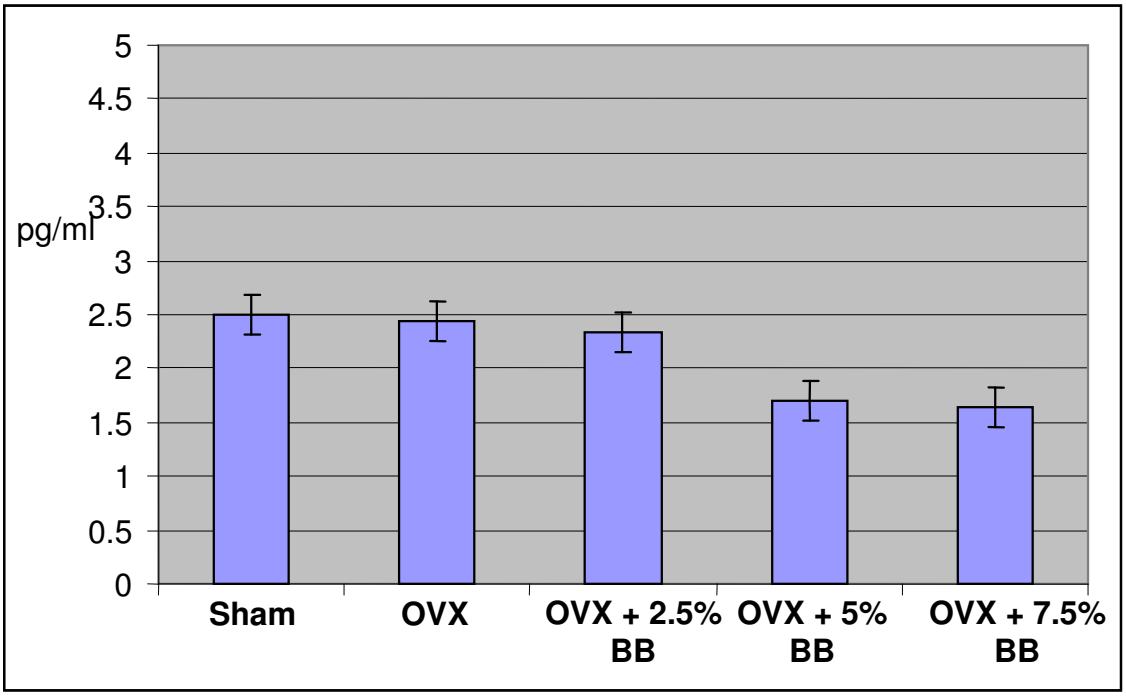
[1] Values are means ± standard errors of the mean, n = 12 or 13 per group.
[2] Bars that do not share the same superscript letters are significantly ($P < 0.05$) different from each other.

Figure 3: The effects of the 90 day intake of three doses of blueberry (BB) on serum levels of lipid hydroperoxide (LPO) of ovariectomized (OVX) rats [1]



[1] Values are means ± standard errors of the mean, n = 12 or 13 per group.

Figure 4: The effects of the 90 day intake of three doses of blueberry (BB) on serum levels of 8-isoprostane of ovariectomized (OVX) rats [1]



[1] Values are means ± standard errors of the mean, n = 12 or 13 per group.

Table 1: Composition of the experimental diet

Ingredients	Sham	Ovx	Ovx+2.5% blueberry	Ovx+5.0% blueberry	Ovx+7.5% blueberry
			g/kg diet		
Blueberry	0	0	25	50	75
Carbohydrate					
Total	720.7	720.7	720.7	720.7	720.7
Cornstarch	466	466	443.6	421.5	399.4
Sucrose	100	100	100	100	100
Dextrinized Corn Starch	155	155	155	155	155
Blueberry provides 88.4% carbohydrate	0	0	22.1	44.2	66.3
Protein					
Total	140	140	140	140	140
Casein	140	140	139.1	138.2	137.3
Blueberry 3.53% protein	0	0	0.9	1.8	2.7
Fat					
Total	40	40	40	40	40
Soybean Oil	40	40	39.5	39	38.5
Blueberry provides 1.91% fat	0	0	0.5	1	1.5
Fiber					
Total	50	50	50	50	50
Cellulose	50	50	48.9	47.8	46.7
Blueberry provides 4.32% fiber	0	0	1.1	2.2	3.3
Vitamin Mix (AIN 93VX)†	10	10	10	10	10
Mineral Mix‡					
Total	37.5	37.5	37.5	37.5	37.5
Mineral Mix (Ca-P Def)	13.4	13.4	13.4	13.4	13.4
Calcium Carbonate	10	10	9.9	9.8	9.8
Blueberry provides 0.12% calcium			0.03	0.06	0.09
Potassium Phosphate, monobasic (KH2PO4)	13.2	13.2	13	12.9	12.6
Blueberry provides 0.10% phosphorus			0.025	0.05	0.075
Potassium Citrate	0.9	0.9	0.9	0.9	0.9
Sucrose	0	0	0.2	0.4	0.6
Choline Bitartrate	2.5	2.5	2.5	2.5	2.5
L-cysteine	1.8	1.8	1.8	1.8	1.8
Tert-butylhydroquinone	0.008	0.008	0.008	0.008	0.008

The composition of these experimental diets was based on the AIN-93M (Harlan Teklad; Madison,WI).
†The vitamin mixture (TD #94047) obtained from Harlan Teklad (Madison, WI) consisted of (g/kg): nicotinic acid, 3.0; calcium pantothenate, 1.6; pyridoxine HCl, 0.7; thiamin HCl, 0.6; riboflavin, 0.6; folic acid, 0.2; D-biotin, 0.02; vitamin B-12 (0.1% mannitol), 2.5; DL-_-tocopheryl acetate (500 IU/g), 15; vitamin A palmitate (500,000IU/g), 0.8; cholecalciferol (500,000 IU/g), 0.2; phylloquinone, 0.075; and sucrose,974.705.
‡The mineral mixture (TD #79055) obtained from Harlan Teklad (Madison, WI) was a modification of the AIN 76 lacking calcium and phosphorus but with sucrose as a diluent.

Table 2: The effects of three doses of blueberry (BB) on food intake and body and organ weights of ovariectomized (OVX) rats [1,2]

	Sham	**Ovx**				
Blueberry	**0**	**0**	**2.5%**	**5%**	**7.5%**	***P value***
Average food intake (g/day)	13.7 ± 0.29	13.9 ± 0.29	13.9 ± 0.29	14 ± 0.29	14.0 ± 0.29	0.9652
Body weights (g)						
Initial	247.5 ± 4.4	247.9 ± 4.4	249.4 ± 4.3	248.9 ± 4.4	249.37 ± 4.3	0.9968
Final	283.9 ± 5.6[b]	320.0 ± 5.6[a]	317.1 ± 5.4[a]	321.3 ± 5.6[a]	319.9 ± 5.4[a]	<0.0001
Uterus (g)	0.54 ± 0.02[a]	0.13 ± 0.02[b]	0.14 ± 0.02[b]	0.15 ± 0.02[b]	0.14 ± 0.02[b]	<0.0001
Liver (g)	6.10 ± 0.14	5.99 ± 0.14	5.91 ± 0.14	6.07 ± 0.14	6.12 ± 0.14	0.8144
Spleen (g)	0.76 ± 0.03	0.73 ± 0.03	0.73 ± 0.03	0.72 ± 0.03	0.73 ± 0.03	0.9262

[1] Values are means ± standard errors of the mean, n = 12 or 13 per group.

[2] Within a row, values that do not share the same superscript letters are significantly ($P < 0.05$) different from each other.

Table 3: The effects of the 90 day intake of three doses of blueberry (BB) on serum and hepatic lipid parameters of ovariectomized (OVX) rats [1,2]

	Sham	**Ovx**				
Blueberry	0	0	2.5%	5%	7.5%	*P value*
Serum lipids						
Triglycerides (mg/dL)	48.0 ± 2.3	52.7 ± 2.3	52.5 ± 2.2	53.0 ± 2.3	53.2 ± 2.2	0.4668
HDL_Cholesterol (mg/dL)	30.8 ± 1.5[b]	44.0 ± 1.5[a]	46.3 ± 1.4[a]	44.5 ± 1.5[a]	47.5 ± 1.4[a]	<0.0001
Liver lipids						
Total lipid (mg/g liver)	58.6 ± 1.5	59.5 ± 1.5	56.3 ± 1.5	55.6 ± 1.5	56.2 ± 1.5	0.3080
Cholesterol (mg/g liver)	3.46 ± 0.23	3.60 ± 0.23	3.03 ± 0.22	3.29 ± 0.23	3.20 ± 0.22	0.4457

[1] Values are means ± standard errors of the mean, n = 12 or 13 per group.

[2] Within a row, values that do not share the same superscript letters are significantly ($P < 0.05$) different from each other.

Table 4: The effects of the 90 day intake of three doses of blueberry (BB) on serum clinical parameters of ovariectomized (OVX) rats [1],[2]

Blueberry	Sham	Ovx				*P value*
	0	**0**	**2.5%**	**5%**	**7.5%**	
Alkaline phosphatase, mg/dL	35.3 ± 3.0[b]	44.2 ± 3.0[a]	48.7 ± 2.9[a]	50.4 ± 3.0[a]	49.6 ± 2.9[a]	0.0041
Phosphorus, mg/dL	4.9 ± 0.14[a]	4.0 ± 0.14[b]	4.2 ± 0.14[b]	4.1 ± 0.14[b]	4.2 ± 0.14[b]	0.0004
Calcium, mg/dL	10.0 ± 0.08	9.7 ± 0.08	9.7 ± 0.07	9.7 ± 0.08	9.7 ± 0.07	0.0661
Glucose, mg/dL	154.4 ± 6.9[b]	188.8 ± 6.9[a]	173.7± 6.7[a]	180.1± 6.9[a]	180.2± 6.7[a]	0.0129
Blood urea nitrogen, mg/dL	16.4 ± 0.66[a]	13.5 ± 0.66[b]	14.0± 0.63[b]	12.9± 0.66[b]	14.0± 0.63[b]	0.0054
Creatinine, mg/dL	0.59 ± 0.02	0.60 ± 0.02	0.55 ± 0.02	0.59 ± 0.02	0.61 ± 0.02	0.4521
Albumin, g/dL	3.5 ± 0.03[a]	3.2 ± 0.03[bc]	3.1 ± 0.03[c]	3.3 ± 0.03[b]	3.2 ± 0.03[c]	<0.0001
Total Protein, g/dL	6.9 ± 0.06[a]	6.6 ± 0.06[bc]	6.5 ± 0.06[c]	6.8± 0.06[ab]	6.7 ± 0.06[b]	0.0001

[1] Values are means ± standard errors of the mean, n = 12 or 13 per group.
[2] Within a row, values that do not share the same superscript letters are significantly ($P < 0.05$) different from each other.

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APPENDIX

Oklahoma State University
Institutional Animal Care and Use Committee (IACUC)

Protocol Expires: 8/3/2008

Date : Wednesday, March 22, 2006 Animal Care and Use Protocol (ACUP) No: HE061

Proposal Title: Blueberry prevents boneloss in Ovariectomized Rats

Principal
Investigator:

Edralin Lucas
Nutritional Sciences
425 HES
Campus

Reviewed and
Processed as: Full Committee **Modification**

Approval Status Recommended by Reviewer(s) : Approved

Modification request to change the lead PI from Dr. Arjmandi to Dr. Lucas is approved.

Signatures

Dr. Charlotte Ownby, IACUC Chair

Wednesday, March 22, 2006
Date

cc: Department Head, Nutritional Sciences
LAR

Approvals are valid for three calendar years, after which time a request for renewal must be submitted. Any modifications to the research project, course, or testing procedures must be submitted for review and approval by the IACUC, prior to initiating any changes. Modifications do not affect the original approval period. Modification approvals are valid for the duration of the protocol approval (see protocol expiration date). Approved projects are subject to monitoring by the IACUC. OSU is a USDA registered research facility and maintains an Animal Welfare Assurance document with the Public Health Service Office of Laboratory Animal Welfare, Assurance number AA3722-01.

VITA

Christina Sue Evans

Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF BLUEBERRIES ON THE LIPID PROFILE OF OVARIECTOMIZED RATS

Major Field: Nutritional Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in May, 2008.

Experience:

Community Nutrition Education Program, Area Coordinator, Oklahoma State University/ Oklahoma Cooperative Extension Service, Oklahoma City, OK – 07/07 - present
Graduate Teaching Assistant, Department of Nutritional Sciences, Oklahoma State University, Stillwater, OK 01/06 – 5/07
Assistant Study Coordinator, Department of Nutritional Sciences, Oklahoma State University, Stillwater, OK 12/03 – 01/06

Professional Memberships: Oklahoma Dietetic Association, American Dietetic Association

Name: Christina Sue Evans Date of Degree: May, 2008

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: THE EFFECT OF BLUEBERRIES ON LIPID PARAMETERS OF OVARIECTOMIZED RATS

Pages in Study: 55 Candidate for the Degree of Master of Science

Major Field: Nutritional Sciences

Scope and Method of Study:

Cardiovascular disease (CVD) is a leading cause of death in the nation with an associated annual health care cost of approximately $448 billion. In women, risk for CVD drastically increases when they reach menopause, partly due to elevation of cholesterol. With the side effects associated with hormone replacement therapy and other prescription medications, dietary modifications play an important role in reducing cholesterol and other CVD risk factors in postmenopausal women. This study examines the effects of blueberry (BB) on modulating lipid profile in ovariectomized (Ovx) rats. Blueberry is a rich source of phenolic compounds and has a high antioxidant capacity. Sixty-two five-month old female Sprague-Dawley rats were either sham-operated (Sham) or Ovx and randomly assigned to one of five treatment groups (n=12-13/group), Sham +control, Ovx +control, Ovx+ 2.5% BB, Ovx +5.0% BB, or Ovx +7.5%BB.

Findings and Conclusions:

After 90 days of treatment, rats were necropsied and tissue samples were collected. Total cholesterol increased due to Ovx but none of the doses of BB were able to prevent the Ovx- induced rise in serum total cholesterol. Triglycerides and liver cholesterol were not altered by Ovx or dietary treatment. The results of this study indicate that the hypercholesterolemic effects of ovariectomy are not prevented by BB.

ADVISER'S APPROVAL: Dr. Edralin A. Lucas